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# CD and Computational studies on Aβ (1-16) suggests determinants of ligand binding and plausible prevention of metal induced toxicity via Betaine like molecules

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**ABSTRACT**

One of the reasons for the plaque formation in Alzheimer's Disease (AD) is the metal induced aggregation of Aβ(1-42). Its C-terminal hydrophobic residues are generally found inside the membrane; but the exposed regions (1-28) are predominantly ligand interacting and believed to be responsible for onset of aggregation events. Recent evidences have indicated that the smaller fragments of Aβ like (17-28), (1-16) and (1-10) are also produced in presence of secretases and elastase. In this background, the current work focuses upon assessing the binding patterns of the residues contained in the smaller fragments (such as 1-16) with metals like zinc, copper, aluminium, and small molecules like betaine and curcumin, via Circular Dichroism (CD) and computational docking methods. The CD data and *in silico* exercises offer valuable information about the determinants that take part in ligand binding and thus contribute to the wealth of knowledge towards appreciating the triggering events related to aggregation patterns of AD. These results not only provide insights into the mechanism that underlie the formation of toxic fragments, but also suggest design of molecules that could function as plausible breakers of the progression of Alzheimer's disease (AD).

## 1. INTRODUCTION

Neuropathological features of Alzheimer's disease (AD) are characterized by selective neuronal loss, neuronal atrophy, neurofibrillary tangles (NFTs) and neuritic plaques [1]. The neuritic or senile plaques are distributed throughout the cerebral cortex and has a core of amyloid surrounded by neuritis [2]. A significant fraction of the cerebral cortex is made up of the β-amyloid, a short 40–42 amino-acid fragment of the transmembrane protein, which is cleaved from the β-amyloid precursor protein (β-APP) [3] by the activity of secretases [4]. The Aβ comprises of a hydrophobic C-terminal domain, a potential beta-strand forming set of residues, and N-terminal region, all of which has the propensity to form different secondary structures under native conditions [5]. A complete conformational study of different fragments in varying conditions suggests that the peptide conformation varies with respect to pH, temperature and concentration [6].

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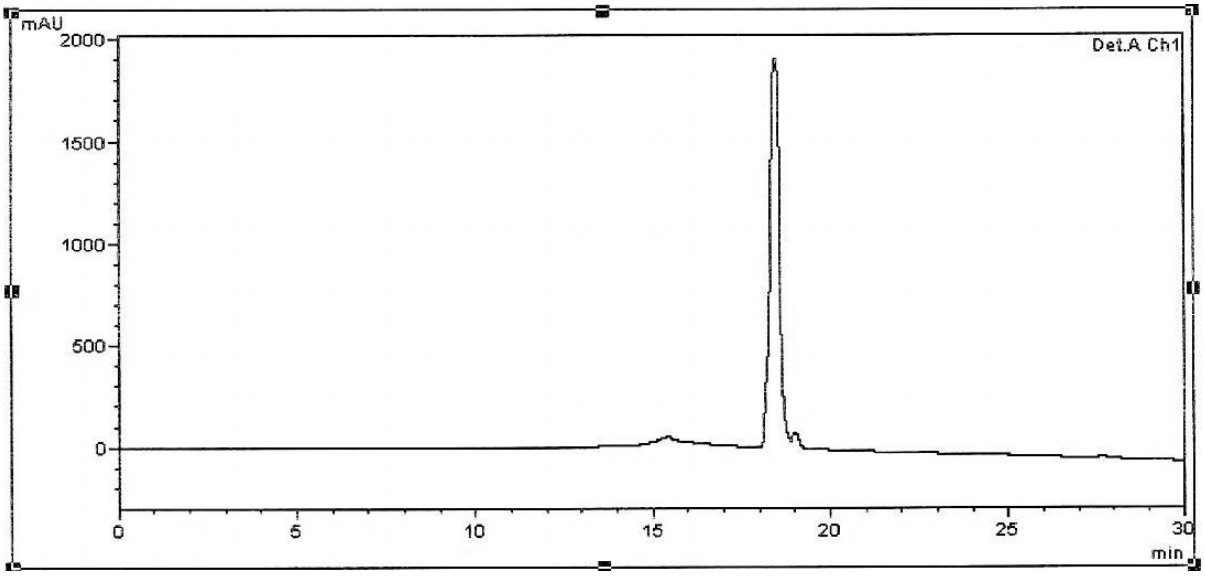
It has been reported that the N terminal region of the Aβ peptide, spanning residues 1-16, lie between the alpha and beta secretase cleavage sites, with the 17-42 not being released into the cell as an amyloidogenic form [7]. This implies that the 1-16 region and specifically the N terminal region is indeed involved in ligand binding and crucial for its neurotoxic properties. Additionally, it has also been documented that the N terminal Aβ(1-16) peptide fragment is further cleaved by elastase into the smaller fragments [8].The literature highlights increased concentrations of metal ions such as, copper, iron, aluminium and zinc in the brains of Alzheimer's disease patients (>0.1mM) [9-12]. There have been substantial evidences that aluminium induces dramatic conformational changes to the peptide [13] which can be reversed by the use of chelators like betaine and borosilicates [14, 15]. Thus, the study of metal binding sites become useful for development of new inhibitors or metal chelators [16], as it would give an insight into their exploitation as potential drug targets. With this background, Circular Dichroism and docking studies on the Aβ(1-16) peptide fragment have been carried out, to decipher the determinants of binding of metals/small molecules/metal chelators, to the peptide, to enable the facilitation of development of effective drugs.




Peak table
Detector A Chl 220nm.

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 18.167 | 495082 | 182329 | 1.330 | 8.435 |
| 2 | 18.474 | 35783089 | 1903025 | 96.135 | 88.035 |
| 3 | 18.999 | 943469 | 76323 | 2.535 | 3.531 |
| Total | | 37221640 | 2161677 | 100.000 | 100.000 |

**Fig. 1:** HPLC profile of Aβ(1-16) peptide.

## 2. MATERIALS AND METHODS

### 2.1 CD studies

The custom made Aβ (1-16) fragment having a sequence, D-A-E-F-R-H-D-S-G-Y-E-V-H-H-Q-K, was purchased from M/s USV Peptides with >95% purity (HPLC grade – refer Fig 1).Various concentrations of metals were prepared in Milli-Q water and used for the interaction studies with peptide. The concentrations of aluminum were at 0.1mM, 0.01mM, 1µM and 0.1µM; the zinc concentrations were at 1mM, 0.1mM, 0.01mM and 1µM; and the copper concentrations were at 10nM, 100nM, 200nM, 400nM and 500nM. The peptide concentration was kept at 0.1mM throughout the ligand interaction studies. The pH was maintained at 6.2 throughout the titrations.

CD spectra were recorded using a JASCO J-715 spectro-polarimeter. Cuvettes with path length of 0.1 cm were used for spectral recording in the range 190 to 250nm with sampling points atevery 1.0nm. The Base line subtraction was done with plain water. The plots were recorded (for 4scans) and raw CD data was converted to molar ellipticity. The data points were collected and secondary structure content determined using the K2D3 software [17]. Varying concentrations of the peptide, namely, 0.01mM, 0.05mM, 0.1mM, 0.5mM and 1mM respectively were prepared, from an initial stock of 1mM.

### 2.2 Docking studies

Molecular docking was carried out using Discovery Studio 3.5 [18]. Cdocker, acharmm-based molecular dynamics (md) simulated-annealing algorithm and a conventional molecular mechanics force field were used for docking analysis [19, 20]. In this docking study, the peptide is kept rigid while the ligands are treated as fully flexible and a final minimization step is used to refine the docked poses. Relevant ligands aluminium, zinc, copper, curcumin, betaine and betaine like molecules namely carnitine, betonicine, prolinebetaine, arsenobetaine, trigonelline, dimethylpropiothetin (DMSP) were retrieved from the NCBI (PubChem Compound database [21] and selected for docking studies with the Aβ(1-16) peptide fragment. The sources and uses of these molecules are elaborated in table 1 [22, 27-31]. As per literature, all these ligands cross the Blood Brain Barrier (BBB) [22, 23, 24]. The 3D coordinates of 1-16 segments were taken from the NMR structure solved by Narayan *et* al [25]. The Aβ peptides were docked to all the ligands by the rigid receptor-flexible ligand docking method [26] and the interactions tabulated.

**Table 1:** Structures and Properties of different ligands used in the study.

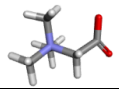
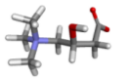
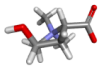
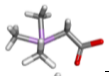
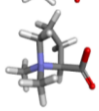
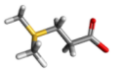
| LIGAND NAME | 3D STRUCTURE | PUBCHEM ID | MOLECULAR FORMULA | MOLECULAR WTG/MOL | SOURCE | USES | REF |
|---|---|---|---|---|---|---|---|
| **Betaine** |  | **247** | **$C_5H_{11}NO_2$** | **117.14634** | Spinach, Beetroot | Anti-inflammatory, Neuroprotection | [22, 27] |
| **Carnitine** |  | **10917** | **$C_7H_{15}NO_3$** | **161.1989** | Meat, yeast | Stimulate gastric and pancreatic secretions and in the treatment of hyperlipoproteinemias. | [22] |
| **Betonicine** |  | **164642** | **$C_7H_{13}NO_3$** | **159.18302** | Yarrow flower | Lowers risk of Cardio vascular diseases | [22] |
| **Prolinebetaine** |  | **115244** | **$C_7H_{13}NO_2$** | **143.18362** | Citrus fruits | Osmoprotectant | [22, 28] |
| **Arsenobetaine** |  | **47364** | **$C_5H_{11}AsO_2$** | **178.06124** | Sea food (shell fish, cod), mushrooms | Not Known | [22, 29-30 ] |
| **Trigonelline** |  | **5570** | **$C_7H_7NO_2$** | **137.13598** | Trigonellafoenum-graecum L. (fenugreek tea and coffee) | Diabetes,Neuroprotective, antimigraine, sedative, memory-improving,antibacterial, antiviral, and anti-tumor activities, | [22, 31] |
| **Dimethy lpropiothetin (DMSP)** |  | **23736** | **$C_5H_{10}O_2S$** | **134.1967** | Algae and higher plants (phytoplanktons) | Anti-ulcer | [22, 32] |

**Table 2:** Fraction of α helix and β sheet in the native Aβ (1-16) with varying ligand concentrations. The concentration of native is maintained at 0.1mM

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Native Aβ (1-16)** | **Concentration of peptide** | | | | | | | | | |
| | **1mM** | | **0.5mM** | | **0.1mM** | | **0.05mM** | | **0.01mM** | |
| | α | β | α | β | **α** | **β** | α | β | α | B |
| | 0.2 | 34.39 | 3.7 | 29.22 | **5.33** | **12.01** | 5.42 | 11.33 | 5.45 | 8.95 |
| **Aluminum** | **Concentrations of metal** | | | | | | | | | |
| | **0.1µM** | | **1µM** | | **0.01mM** | | **0.1mM** | | | |
| | α | β | α | β | α | β | α | β | | - |
| | 8.41 | 28.99 | 2.63 | 40.53 | 2.22 | 42.53 | 1.82 | 47.7 | | |
| **Zinc** | **Concentrations of metal** | | | | | | | | | |
| | **1µM** | | **0.01mM** | | **0.1mM** | | **1mM** | | | |
| | α | β | α | β | α | β | α | β | | - |
| | 8.50 | 12.68 | 6.52 | 13.18 | 5.5 | 18.12 | 5.08 | 19.32 | | |
| **Copper** | **Concentrations of metal** | | | | | | | | | |
| | **10nM** | | **100nM** | | **200nM** | | **400nM** | | **500nM** | |
| | α | β | α | β | α | β | α | β | α | β |
| | 1.66 | 16.64 | 5.49 | 12.40 | 5.73 | 12.68 | 6.71 | 12.39 | 8.96 | 12.33 |
| **Curcumin** | **Concentrations of ligand** | | | | | | | | | |
| | **1µM** | | **0.01mM** | | **0.1mM** | | | | | |
| | α | β | α | β | α | β | | | | |
| | 5.46 | 12.86 | 5.48 | 7.91 | 5.48 | 7.78 | | | | |
| **Betaine** | **concentrations of ligand** | | | | | | | | | |
| | **1µM** | | **0.01mM** | | **0.1mM** | | **1mM** | | | |
| | α | β | α | β | α | β | α | β | | |
| | 2.2 | 11.87 | 1.71 | 9.97 | 1.64 | 7.77 | 1.26 | 7.62 | | |
| **Aluminium and Betaine** | **Concentration of Al and Peptide at 0.1mM** | | **Concentration of Betaine** | | | | | | | |
| | | | **1µM** | | **0.01mM** | | **0.1mM** | | | |
| | α | β | α | β | α | β | α | β | | |
| | 1.82 | 47.7 | 63.02 | 0.09 | 72.97 | 0.02 | 88.69 | 0.01 | | |

## 3. RESULTS AND DISCUSSION

### 3.1. Concentration Dependent Studies

The CD spectra of the native Aβ (1-16) peptide at various concentrations, is as shown in Fig. 2, while the derived secondary structure values are tabulated in Table 2. The graphs highlight that, the peptide has a tendency for aggregation with increase in concentration, which is noted by the increase in the beta sheet content and decrease in alpha helical values. Thus it is evident that the Aβ (1-16) has a propensity to form beta sheets at higher

concentrations and assumes a more random coil structure as the concentrations decrease, which is in accordance with earlier studies [6].

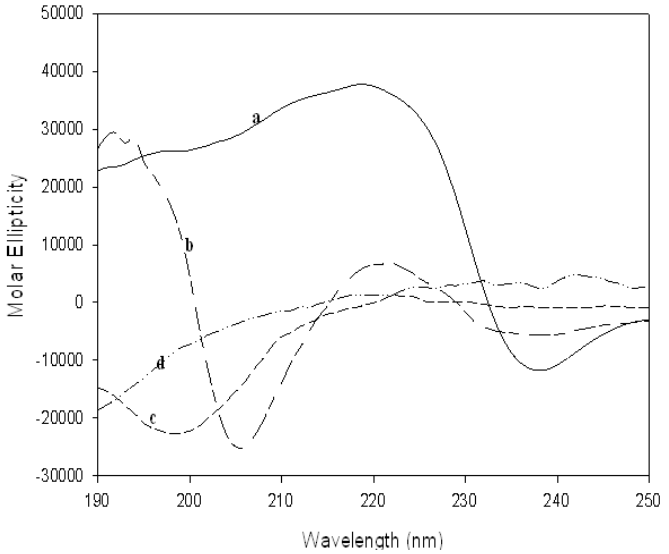



**Fig. 2:** CD Spectra of Native Aβ(1-16) at different concentrations a) 1mM; b) 0.5mM; c) 0.1mM; d) 0.05mM; e) 0.01mM. The units for Molar Ellipticity are given in deg. $cm^2$ $dmol^{-1}$

### 3.2 Interaction studies with ligands

An overlay of the CD spectra of Aβ(1-16) peptide fragment with ligands like aluminum, copper, zinc, curcumin and betaine, at respective concentrations is shown in Fig. 3, which depict maximum variations with reference to native conformation. The corresponding secondary structure values are given in Table 2. Our results highlight that, the negative band was around 190nm to 198 nm (Fig. 3), indicating marked changes in the peptide conformations. It is also noted that the maximum changes in the negativity occurs at 198nm on addition of copper and aluminium as depicted in spectra c and f respectively. These results also indicate that the ordering of peptide is maximum on addition of copper and aluminium. The fraction of α helix and β sheet contents indicated in Table 2, suggest aluminium induced aggregation, similar to the observations made in various literatures [13, 15, 33]. Exley *et al.*, (1993) have mentioned that at a higher concentration of aluminium, there is a loss of alpha helical content in favor of beta turns and random coil. This is in accordance with our results as well, where notable increase in the beta sheet content is seen from the table, probably reiterating the fact that aluminium is a potential neurotoxic metal. It is also seen that the molar ellipticity increases at 198nm on addition of zinc and curcumin (spectra b and e). It is interesting to note that spectra d (betaine-peptide complex) is closer to native, which indicates that betaine is effective in maintaining the native like geometries. In order to explore the conformational reversal by betaine, as reported in earlier work [13], the metal-bound forms of Aβ(1-16) at 0.1mM aluminium were again titrated with varying concentrations of betaine. It was inferred from spectra (g) that on addition of betaine to the peptide aluminium complex, the changes in the spectra and thus the conformation of the peptide were significant. Further, it was notable that a peak appeared around 220-225nm for the peptide interactions with zinc, copper, curcumin and betaine, which could be attributed to the backbone geometries.

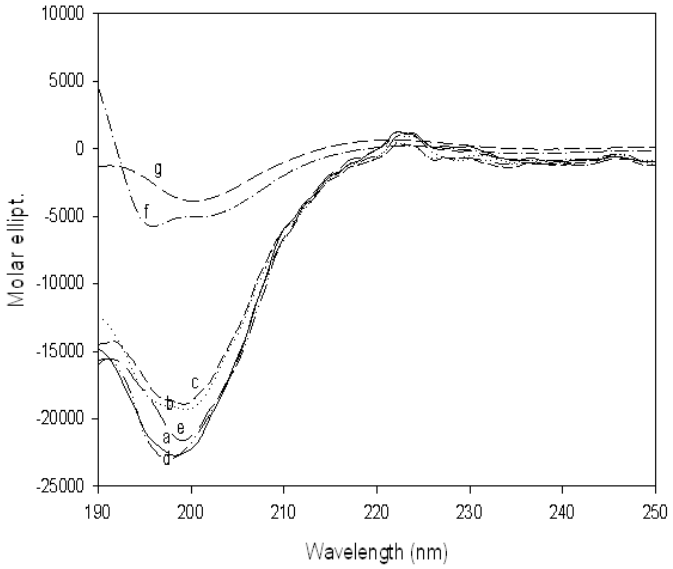



**Fig. 3:** Spectra of a) 0.1mM Native peptide; b) 0.1mM peptide with 1mM $Zn^{2+}$; c) 0.1mM peptide and 10nM $Cu^{2+}$; d)0.1mM peptide and 0.01mMbetaine; e)0.1mM peptide and 0.1mMcurcumin; f)0.1mM peptide with 0.1mM $Al^{3+}$; g) 0.1mM peptide with 0.1mM$Al^{3+}$ and 0.1mMbetaine. The units for Molar Ellipticity are given in deg. $cm^2$ $dmol^{-1}$

### 3.3 Docking studies with ligands

The docking of ligands to the Aβ(1-16) fragment is indicated in Fig 4 and tabulated in Table 3, which shows the potential binding determinants to various ligands. Aluminium appear to bind to residues A2, F4, R5, G9, Y10 and Q15 respectively, while, the residues interacting with zinc were Y10, E11, H13 and Q15. Similarly, the amino acids S8, E11, H13, H14 and Q15 network with copper. In case of curcumin, the interacting determinants were found to be D1, Y10, E11, V12, H13, H14 and Q15 with curcumin; while, only F4 and R5 seem to bind with betaine. Interestingly, the residues E11 and Y10 seem to interact with betaine when docked to the aluminium bound peptide. The interaction of betaine with Aβ(1-16) indicates the involvement of Y10 and E11 residues in the aluminium bound form, rather than F4 and R5 (as in aluminium unbound form).

This suggests that the ligand betaine binds to the peptide in the alternate sites and probably holds the conformation of the tail of the peptide (1-16) in the healthy alpha-helical conformation; than allowing the toxic beta-sheet like conformation, due to binding of aluminium. The strengths of interactions are tabulated in Table 3.

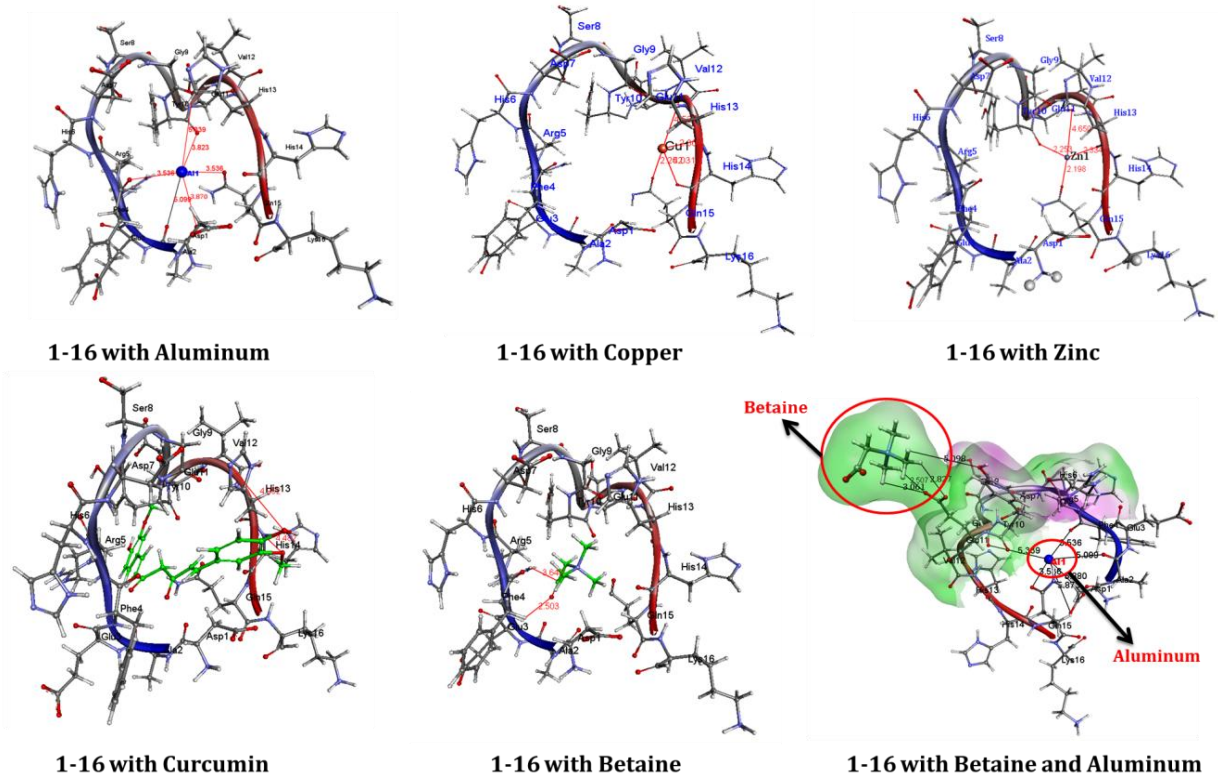



**Fig. 4:** Docking interactions of Aluminum, Zinc, Copper, Betaine, and Curcumin Ligands with Aβ (1-16) fragment.

**Table 3:** Residues of Aβ (1-16) fragments interacting with the ligands within 5.0 Å indicating the relative Strength of interactions. The number of interacting residues is given in parenthesis.

| Residues Involved in ligand interactions (within 5Å) | | Aluminum | Zinc | Copper | Betaine | Curcumin | Betaine with Aluminium bound |
|---|---|---|---|---|---|---|---|
| For 1 -16 | D1 | - | - | - | - | | |
| | A2 | 4.89 | | | | | |
| | F4 | 3.536 | - | - | 4.57 | - | |
| | R5 | 3.83 | - | - | 2.73 | - | |
| | S8 | - | - | 2.367 | - | - | |
| | G9 | | | | | | |
| | Y10 | 3.283 | 2.253 | - | - | 2.61 | 4.63 (Betaine) |
| | E11 | - | 4.650 | 4.527 | - | 4.73 | 2.87 (Betaine)<br>3.06 (Betaine)<br>2.50 (Betaine) |
| | V12 | - | - | - | - | 3.16 | |
| | H13 | - | 2.334 | 2.309 | - | 3.13 | |
| | H14 | - | - | 4.031 | - | 2.68 | |
| | Q15 | 3.53 | 2.198 | 2.252 | - | 4.07 | |
| Strength of interaction | | Very Strong (5) | Strong (4) | Very Strong (5) | Moderate (2) | Very Strong (6) | Strong (4) |

### 3.4 Docking studies with Betaine like molecules

Our Docking results highlight that the Betaine like molecules interact strongly with the peptide. The interacting residues are indicated in Table 4 and the docked poses are depicted in Fig 5. It is interesting to observe that, in the absence of aluminium, the site of docking coincides with that of the metal. However, in the presence of aluminum (metal bound complex), the interaction mode shifts indicating that, though the aluminium interaction site did not change, the betaine like molecules appear to interact strongly in an alternate site, as shown in Fig 6.Our results indicate that arsenobetaine shows stronger interaction with the peptide-Aluminium complex, than the native peptide. It is also observed that Q15 plays important roles in binding of betaine like molecules under native conditions, whereas H13 seems to interact strongly in the case of complexes involving peptide-aluminium-betaine analogues (refer Tables 4, 5).Further, in the presence of aluminium, the strength of interactions and the binding modes of thesebetaine like systems varied. The interacting residues and their strengths are highlighted in Table 5. Taken together we conclude that these molecules could serve as potential metal chelators and plausible therapeutic agents against aluminium induced toxicity in AD.

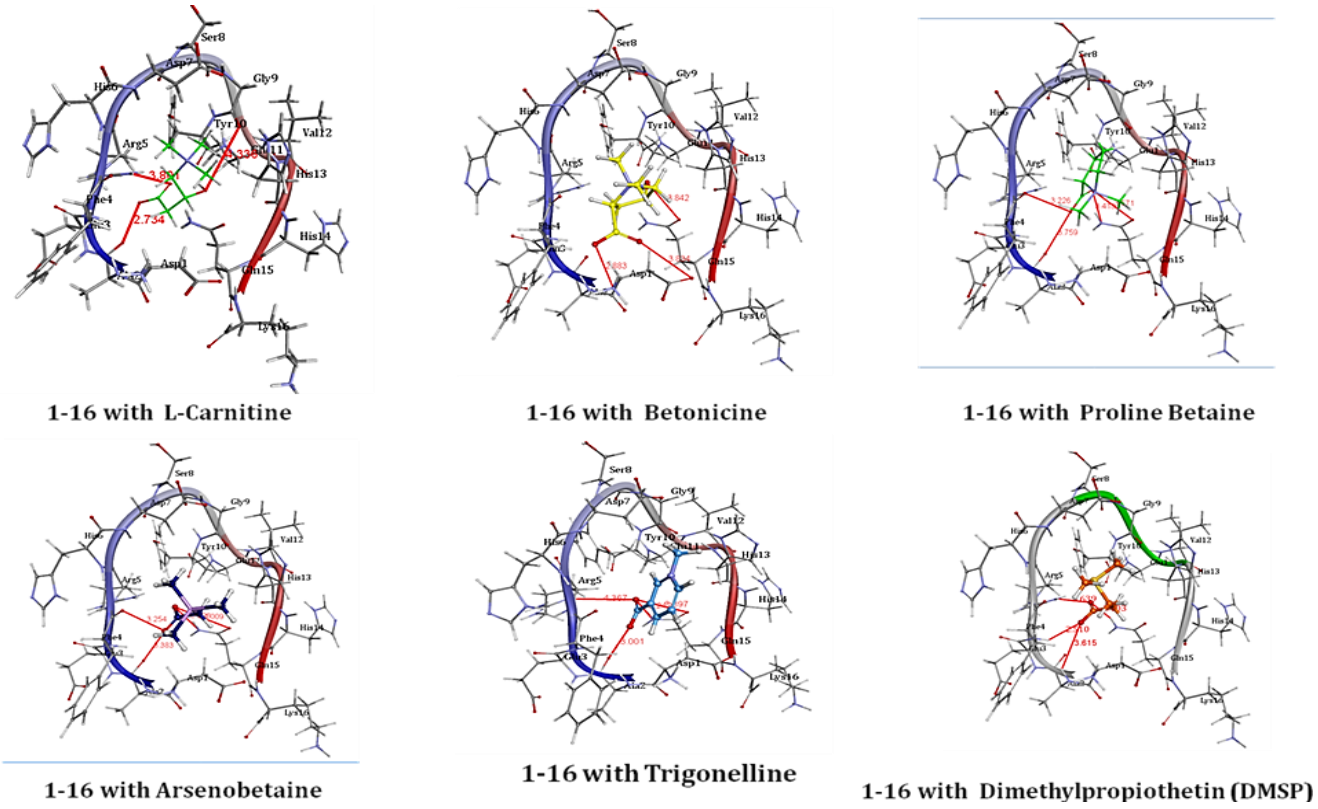



**Fig. 5:** Docking interactions of Betaine like molecules with Aβ(1-16) fragment.

**Table 4:** Residues of Aβ(1-16) fragments interacting with betaine and its analogues within 5.0 Å indicating the relative Strength of interactions.The number of interacting residues is given in parenthesis.

| Residues Involved in ligand interactions (within 5Å) | | Betaine | L-Carnitine | Betonicine | ProlineBetaine | Arsenobetaine | Trigonelline | Dimethyl propiothetin (DMSP) |
|---|---|---|---|---|---|---|---|---|
| For 1 -16 | D1 | | - | 3.834<br>2.833 | - | - | | |
| | A2 | | 2.75 | | 3.759 | 3.383 | 3.001 | 3.615 |
| | E3 | | | | 3.226 | | | |
| | F4 | 4.57 | | 3.902 | - | 3.254 | - | 2.510 |
| | R5 | 2.73 | 3.80 | - | - | | 4.367 | 3.639 |
| | S8 | | - | - | | - | - | |
| | G9 | | | | | | | |
| | Y10 | | 4.336 | | | - | | |
| | E11 | | - | | | - | | |
| | V12 | | - | - | | - | | |
| | H13 | | - | | | - | | |
| | H14 | | - | - | | - | | |
| | Q15 | | | 3.842 | 3.418<br>3.571 | 3.228<br>4.009 | 3.242<br>3.897 | 2.013 |
| Strength of interaction | | Moderate (2) | Moderate (3) | Strong (4) | Strong (4) | Strong (4) | Strong (4) | Strong (4) |

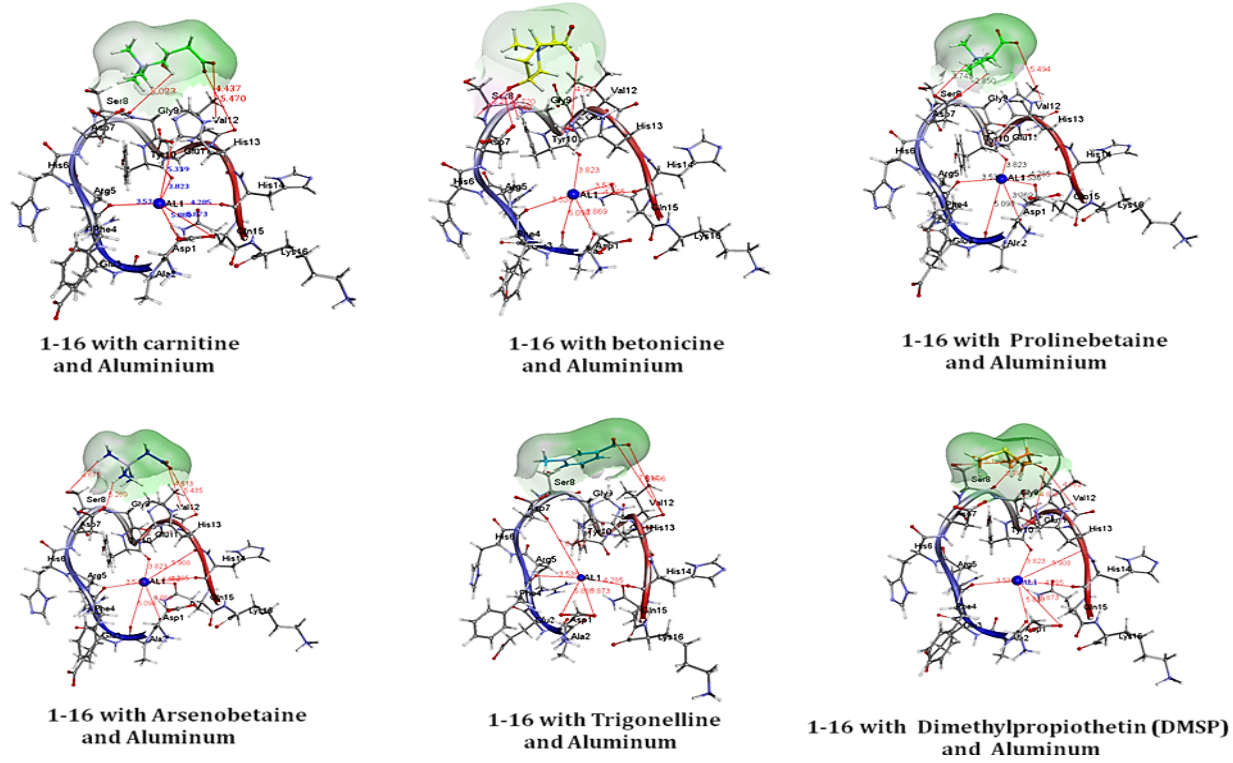



**Fig. 6:** Docking interactions of Aluminium and Betaine like molecules with Aβ (1-16) fragment.

**Table 5:** Residues of Aβ (1-16) fragments interacting with Betaine and its analogues complexed with aluminium within 5.0 Å indicating the relative Strength of interactions. The number of interacting residues is given in parenthesis.

| Residues Involved in ligand interactions (within 5Å) | | L-Carnitine with $Al^{3+}$ | Betonicinew ith$Al^{3+}$ | ProlineBetainew ith$Al^{3+}$ | Arsenobetainew ith$Al^{3+}$ | Trigonelline with$Al^{3+}$ | Dimethylpropiot hetin (DMSP) with$Al^{3+}$ | Betaine with $Al^{3+}$ |
|---|---|---|---|---|---|---|---|---|
| For 1 -16 | D1 | - | - | - | - | - | - | - |
| | A2 | - | - | - | - | - | - | - |
| | E3 | - | - | - | - | - | - | - |
| | F4 | - | - | - | - | - | - | - |
| | R5 | - | - | - | - | - | - | - |
| | D7 | - | 2.14 | 3.7<br>2.7 | 3.7 | - | - | - |
| | S8 | 4.0 | 3.21 | - | - | - | 3.4 | - |
| | G9 | - | 2.71 | - | - | - | 4.96 | - |
| | Y10 | - | - | - | - | - | - | 4.63<br>2.87 |
| | E11 | - | - | - | - | - | - | 3.06<br>2.50 |
| | V12 | 4.58 | - | - | 4.3 | - | - | - |
| | H13 | 4.05 | 4.5 | 4.8 | 4.6 | 3.53 | 4.06 | - |
| | H14 | - | - | - | - | - | - | - |
| | Q15 | - | - | - | 3.228<br>4.009 | 3.242<br>3.897 | - | - |
| Strength of interaction | | Moderate (3) | Strong (4) | Moderate (3) | Very Strong (5) | Moderate (3) | Moderate (3) | Strong (4) |

## 4. CONCLUSIONS

While the native conformation of the peptide is unstructured and near random coil, it is likely that it adopts an ordered structure when complexed with metals. The changes were expectedly predominant in the case of aluminium. Though the secondary structure content did not change drastically, there is an indication that the ligands bind to the peptide strongly at higher concentrations, and undergo substantial conformational changes. The most interesting change was exhibited in the copper titration where the metal did not induce formation of secondary structure elements and appeared to stall the aggregation process. The role of copper as a possible neuroprotective metal could be better exploited through further investigations. These results are in

accordance with earlier literature suggesting the neuroprotective nature of copper at sub micromolar levels [34]. Our results with zinc also propose that at a lower concentration of metal, the peptide has the propensity to form alpha helical structural elements. It indicates that lower concentrations of zinc may be neuroprotective, while is rendered gradually toxic at higher concentrations, thus correlating with earlier literature [35]. However, it is noteworthy to cautiously suggest that these estimates of the secondary structure content from the CD spectral changes are only indicative of the possible secondary structural changes, and cannot be taken on an absolute scale. Betaine and curcumin also appear to bind to the peptide and decrease the beta sheet content (up to 40%), which suggest their use as potential therapeutic agents. Interestingly, betaine appears to act as a possible metal chelator, and supposedly reducing the aluminium induced toxicity of AD. This work provides due insights into the probable use of betaine and betaine like molecules as potential drug candidates for AD. Arsenobetaine shows stronger interaction with the peptide in presence of aluminium, and this provides vital clues towards designing effective drugs/inhibitors specifically for aluminium induced neurotoxicity. Since betaine like molecules are known to be present in our diet [22], we conclude that these could offer potential natural remedies for AD. Similarly, curcumin like molecules [36] could also be exploited towards designing lead compounds to tackle the onset of AD.

Collectively, these results offer clues towards the binding nature of metals and small molecules to the Aβ(1-16) peptide fragment, and exhibits promising leads towards the development of potential therapeutics for AD. It is evident from literature that the current drugs like Donepezil, Mementine and cholinesterase inhibitors target the acetylcholine esterase, which appears to slow down the progression of the disease [37]. However, these are accompanied by inherent side effects [38]. Interestingly there is currently no molecule which prevents the oligomerization/aggregation of the Aβ peptide and thus curtails neuronal death. The molecules under consideration in our study are targeted towards disrupting the Aβ aggregates, which could possibly offer plausible cure for the disease. The *in silico* exercises strengthen the need for elucidation of high resolution NMR structures of these peptides with metal and small molecules/ligands, to propose the design of efficacious inhibitors.

## 5. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## 7. REFERENCES

1. Bergeron C. Alzheimer's Disease Neuropathological Aspects. *Can J Vet Res*. 1990; 54 (1):58–64.
2. Palmer M. Neurochemical Studies of Alzheimer's Disease. *Neurodegeneration*. 1996; 5(4): 381–391.
3. Munoz D.G and Feldman H Causes of Alzheimer's disease. *CMAJ*. 2000; 162(1): 65–72.
4. Kosik K. S. The Alzheimer's disease sphinx: a riddle with plaques and tangles. *J Cell Biol.* 1994; 127(6): 1501–1504.
5. Serpell L. C Alzheimer's amyloid fibrils: structure and assembly. *Biochimica et biophysica acta.* 2000; 1502(1):16–30.
6. Barrow C. J, Yasuda A, Kenny PT, Zagorski MG Solution Conformations and Aggregational Properties of Synthetic Amyloid Peptides of Alzheimer's Disease Analysis of Circular Dichroism Spectra. *Journal of molecular biology*, 1992; 225(4):1075–1093.
7. Gowing E, Roher A E, Woods A S, Cotter R J, Chaney M, Little S P, Ball M J Chemical characterization of A beta 17-42 peptide, a component of diffuse amyloid deposits of Alzheimer's disease. *The Journal of biological chemistry*, 1994; 269(15):10987–90.
8. Damante C A, Ösz K, Nagy Z, Grasso G, Pappalardo G, Rizzarelli E, Sóvágó I. Zn $^{2+}$ 's Ability to Alter the Distribution of Cu $^{2+}$ among the Available Binding Sites of Aβ(1-16)-Polyethylenglycol-ylated Peptide: Implications in Alzheimer's Disease. *Inorg Chem.* 2011; 50(12): 5342–5350.
9. Lovell M A, Robertson J D, Teesdale W J, Campbell J L, Markesbery W R. Copper, iron and zinc in Alzheimer's disease senile plaques. *J Neurol Sci.* 1998; 158(1):47–52.
10. Bush A.I The metallobiology of Alzheimer's disease. Trends *Neurosci*. 2003; 26(4):207–14.
11. Schetinger M.R.C. Bonan C.D. Morsch V.M. Bohrer D. Valentim L.M. and Rodrigues S.R. Effects of aluminum sulfate on delta-aminolevulinatedehydratase from kidney, brain, and liver of adult mice *Brazilian Journal of Medical and Biological Research*. 1999;(32):761-766.
12. Strozyk D, Launer LJ, Adlard PA, Cherny RA, Tsatsanis A, Volitakis I, Blennow K, Petrovitch H, White LR, Bush AI. Zinc and copper modulate Alzheimer Abetalevels in human cerebrospinal fluid .*Neurobiol Aging*. 2009; 30(7): 1069-77
13. Ramakrishna T, Vatsala S, Shobi V, Sreekumaran E, Madhav TR, Ramesh J, EaswaranK R K. Betaine reverses toxic effects of aluminium: implications in Alzheimer's disease (AD) and ADlike pathology. *Current Science*, 1998; 75(11): 1153–1161.
14. Ramakrishna T, Vatsala S, Madhav TR, Sreekumaran E, Ramesh J, Easwaran KRK. Conformational Change in b-amyloid Peptide [1-40] with Aluminium: Reversal by Borate. *Alzheimer's Research*, 1997; 3(5), pp. 223-226.
15. Fasman G. D. Perczel A. and Moore C. D. Solubilization of beta-amyloid-(1-42)-peptide: reversing the beta-sheet conformation induced by aluminum with silicates. *Proc Natl Acad Sci U S A*, 1995*;* 92(2):369–71.
16. Curtain C. C. Barnham K. J. and BushA. I. Aβ Metallobiology and the Development of Novel Metal-Protein Attenuating Compounds (MPACs) for Alzheimer's Disease. *Current Medicinal Chemistry*, 2003; 3(4): 309–315.
17. Caroline Louis-Jeune, Miguel A. Andrade-Navarro, Carol Perez-Iratxeta Prediction of protein secondary structure from circular dichroism using theoretically derived spectra. *Proteins: Structure, Function, and Bioinformatics*. 2012; 80(2):374–381.
18. Discovery studio (DS) (Discovery Studio 3.5, Accelrys Inc. San Diego, California, (http://www.accelrys.com/). USA).

19. Momany, F. A.; Rone, R. J Validation of the general purpose QUANTA 3.2/CHARMm force field. *Comp. Chem*.1992;(13): 888-900.
20. Krammer A. Kirchhoff P. D. Jiang X. Venkatachalam C. M. Waldman, M.LigScore: a novel scoring function for predicting binding affinities. *J. Mol. Graph. Model*, 2005; 23: 395-407.
21. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA,Wang J, Yu B, Zhang J, Bryant SH. PubChem Substance and Compound databases. *Nucleic Acids Res*. 2015; 4(44):1202-13.
22. deZwart, F. Slow, S. Payne, R. L. George, P. Gerrard, J. Chambers, S. Glycine betaine and glycine betaine analogues in common foods. *Food Chemistry*. 2003; 83: 197 – 204.
23. Mroczkowska JE, Roux FS, Naleçz MJ, Naleçz KA Blood-brain barrier controls carnitine level in the brain: a study on a model system with RBE4 cells . *BiochemBiophys Res Commun*. 2000;7, 267(1):433-7.
24. Liana Fattore and Walter Fratta Front. Beyond THC: the new generation of cannabinoid designer drugs *Front BehavNeurosci.* 2011; 5(60):1-12.
25. Narayan P., Krishnarjuna B., Vishwanathan V., Jagadeesh Kumar D., Babu S., Ramanathan KV, Easwaran KRK, Nagendra HG and Raghothama S. Does aluminium bind to histidine? An NMR investigation of amyloid β12 and amyloid β16 fragments, *Chem biol drug des.* 2013; 82(1): 48–59.
26. Koska J, Spassov VZ, Maynard AJ, Yan L, Austin N, Flook PK, Venkatachalam CM Fully automated molecular mechanics based induced fit protein-ligand docking method .*Chem Inf Model*. 2008; 48(10): 1965-73.
27. Craig SA. Betaine in human nutrition. *Am J ClinNutr*. 2004; 80(3): 539-49.
28. Heinzmann SS, Brown IJ, Chan Q, Bictash M, Dumas ME, Kochhar S, Stamler J, Holmes E, Elliott P, Nicholson JK. Metabolic profiling strategy for discovery of nutritional biomarkers: prolinebetaine as a marker of citrus. *Am J ClinNutr*. 2010; 92: 436–43.
29. Molina M., Ulvena SM, Dahlb L., Telle-Hansena VH, Holcka M., Skjegstada G., Ledsaaka O., Slothb JJ, Goesslerd W, Oshauga A, Alexandere J, Fliegelb D, Ydersbondf TA, Meltzere HM. Humans seem to produce arsenobetaine and dimethylarsinate after a bolus dose of seafood *Environmental Research.* 2012; 112: 28–39.
30. John S. Edmonds, Kevin A. Francesconi, Jack R. Cannon, Colin L. Raston, Brian W. Skelton and Allan H. White. Isolation, crystal structure and synthesis of arsenobetaine, the arsenical constituent of the western rock lobster *panuliruslongipescygnus George*. *Tetrahedron Letters.* 1997; 18:1543–1546.
31. Zhou J1, Chan L, Zhou S, Trigonelline: a plant alkaloid with therapeutic potential for diabetes and central nervous system disease *Curr Med Chem*. 2012; 19(21):3523-31.
32. Duane C. Yoch Dimethylsulfoniopropionate: Its Sources, Role in the Marine Food Web, and Biological Degradation to Dimethylsulfide. *Appl. Environ. Microbiol*,2002; 68(12):5804-5815.
33. ChenY. R. HuangH. B. Chyan C. L. Shiao M. S. LinT. H. and Chen Y. C. The effect of Abeta conformation on the metal affinity and aggregation mechanism studied by circular dichroism spectroscopy, *J Biochem.* 2006; 139(4):733–40.
34. Syme C. D. Nadal R. C. Rigby S. E. J. and Viles J. H. Copper binding to the amyloid-beta (Abeta) peptide associated with Alzheimer's disease: folding, coordination geometry, pH dependence, stoichiometry, and affinity of Abeta-(1- 28): insights from a range of complementary spectroscopic techniques. *J Biol Chem*. 2004: 279(18): 18169–77.
35. Syme C. D. and Viles J. H. 'Solution 1H NMR investigation of $Zn^{2+}$ and $Cd^{2+}$ binding to amyloid-beta peptide (Abeta) of Alzheimer's disease. *BiochimBiophysActa. 2006;* 1764(2): 246–56.
36. Alok Vyas, Prasad Dandawate, Subhash Padhye, Aamir Ahmad, and Fazlul Sarkar Perspectives on New Synthetic Curcumin Analogs and their Potential Anticancer Properties *Curr Pharm Des*. 2013;19(11):2047–2069.
37. Owen RT,Memantine and donepezil: a fixed drug combination for the treatment of moderate to severe Alzheimer's dementia, *Drugs Today (Barc)*, 2016,52(4):239-48.
38. Buckley JS, Salpeter SR A Risk-Benefit Assessment of Dementia Medications: Systematic Review of the Evidence. *Drugs Aging*, 2015; 32(6):453-67.

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